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Accumulation and detoxification of metals and arsenic in tissues of cattle (*Bos taurus*), and the risks for human consumption

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HIGHLIGHTS

- Cadmium induced metallothionein in kidney while Zn induced metallothionein in liver.
- For Mn and Cd a significant part of the uptake happens via the lungs.
- 40% of the livers and 85% of the kidneys exceeded the European limit for cadmium.
- A person of 70 kg should not eat more than 150 g bovine meat per day.

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ABSTRACT

The aim of this study was to investigate metal accumulation and detoxification processes in cattle from polluted and unpolluted areas. Therefore dairy cows from farms and free ranging Galloway cows from nature reserves were used as study animals. The concentrations of Ag, Cd, Pb, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn and As were determined in muscle, kidney, liver and lungs of cattle from polluted and reference areas in Belgium. In kidney and liver also the metallothionein concentrations were measured. For Ag, Mn, Co, Cu, Zn and As the concentrations in the different tissues were significantly higher in the sampled Galloways than in the sampled dairy cows. On the other hand Cd and Pb were significantly higher in tissues of both cattle breeds from polluted sites. Cadmium seemed to be the most important metal for metallothionein induction in kidneys whereas Zn seemed to be the most important metal for the induction of metallothionein in the liver. This study also suggested that only for Mn and Cd a significant part of the uptake occurs via the lungs. Although in muscle none of the Cd and Pb levels exceeded the European limits for human consumption, 40% of the livers and 85% of the kidneys of all examined cows were above the European limit for cadmium. Based on the existing minimum risk levels (MRLs) for chronic oral exposure, the present results suggested that a person of 70 kg should not eat more than 150 g cow meat per day because of the Cr levels in the muscles.

1. Introduction

As a result of both historical and ongoing industry, mining and agricultural activities, metal pollution worldwide is still one of the most important environmental threats. The metalloid arsenic (As) and the metals lead (Pb), cadmium (Cd), copper (Cu) and silver (Ag) are respectively classified as numbers 1, 2, 7, 125 and 217 on the priority list of the most hazardous substances in the environment by the Agency for Toxic Substances and Diseases Registry (ATSDR, 2011). Some metals such as Cd are mobile in plants and may become concentrated in leaves. Therefore, grazing animals may potentially be highly exposed to these elements via ingestion of polluted soil, vegetation, drinking water and possibly also via inhalation (Madejón et al., 2009; Reglero et al., 2008). Knowledge of metal concentrations in livestock is therefore important for assessing the effects of these pollutants on domestic animals, as well as the potential risk from human consumption. The effects of metals on animal health can partially be controlled by the animal's detoxification mechanisms. The major homeostatic metal control mechanisms of cattle are changes in absorption rate, urinary excretion, tissue deposition in harmless or mobile reserve forms, secretion into milk and endogenous excretion via feces. The importance of each of these detoxification mechanisms varies greatly among the elements (Miller, 1975). In the liver and kidney Cd, Cu, Zn and to a lesser extend Pb may induce and bind metallothioneins (MTs) (Nordberg et al., 1994). These MTs are small cysteine-rich metal binding proteins with a low molecular weight and are involved in the binding, transport and detoxification of excessive Cd and other metals (Nordberg and Nordberg, 2009; Öhrvik et al., 2007). They play

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an important role in the protection of cells against an excess of essential metals but may also detoxify non-essential metals (Roesijadi, 1996). Therefore, they are often measured in tissues as an important biomarker for metal toxicity. Although many laboratory studies investigated the induction of MTs in rodents, few field studies investigated the relationship between metal burden and MT levels in vertebrates (Fritsch et al., 2010; López-Alonso et al., 2005; Rogival et al., 2007; Vanparys et al., 2008). Moreover, studies that investigate the relationship between accumulated metal levels and MT levels in tissues of cattle are scarce (López-Alonso et al., 2005; Olsson et al., 2001).

The non-essential metals that have been studied most frequently are Cd and Pb. From the essential metals Cu and Zn are most studied. Cadmium is considered to be one of the most toxic metals in the environment due to its wide range of organ toxicity and its long elimination half-life of 10–30 years (Nawrot et al., 2006). Respiratory and digestive systems are both involved in Cd absorption. Approximately 10 to 50% of Cd fumes are absorbed by the respiratory system. After inhalation Cd accumulates in the olfactory bulb (Sunderman, 2001) and in the lungs where it can pass through alveolar cells into the blood (Bressler et al., 2004). The exact mechanisms for Cd absorption aren't yet fully elucidated but it may be bound to chelators such as glutathione or cysteïne. Being a non-essential element, Cd is unlikely to enter the body by a Cd specific transport mechanism but many studies have suggested that Cd crosses various membranes utilizing the transport mechanisms of other elements such as calcium (Martelli et al., 2006).

The aim of this study was to investigate how trace elements (e.g. essential metals, non-essential metals, the metalloid arsenic) are accumulated and detoxified in cattle. The influence of cattle breed (Holstein, Galloway) and management practice (agricultural, free-range) on metal tissue concentrations and MT were investigated. Possible accumulation via the air-lung pathway was studied for each metal. Different methods for the preparation of the samples (homogenized versus not homogenized) were compared in order to be able to select the best methods for future studies. Possible relationships between metals and MT concentrations in the organs were tested and a histopathological investigation of the different tissues was conducted in order to investigate the detoxification capacity and possible damage of the organs. Finally the potential risk for human consumption of meat from cattle grazing at historically contaminated sites was assessed.

2. Materials and methods

2.1. Study animals

In this study a distinction was made between agricultural dairy cows and free ranging Galloway cows. All sampled animals were slaughtered for human consumption. Dairy cows were used instead of beef cattle because they are usually raised to over 18 months (Waegeneers et al., 2008). The sampled dairy cows were almost exclusively Holstein cows because they are most commonly used in Belgium. During spring and summer dairy cows graze on pastures while during autumn and winter they are kept in stables and are fed different types of feed. For this study, only females more than 5 years old were selected. The selection was made in the slaughter house on the day of slaughter and was based on the information of the cow's passport. Each bovine animal in Belgium is obligatory marked by an ear tag with a unique identification number (sanitel number) and has a bovine passport that contains information of the date of birth, sex and breed of the animal and of the farm where the animal resided (Table S1).

Free ranging Galloways were also investigated because they are the most common cow breed used for grazing management of nature reserves in Belgium. Only animals that resided year round in the same reserve for at least 4 years were selected. They lived in semi-wild conditions and were not fed by humans. Because few Galloways from nature reserves are slaughtered, males and oxen were also included in order to obtain a sufficient number of samples (Table S1).

Only farms and nature reserves were selected in regions from which the pollution status (polluted or reference) was known. The selection of polluted regions (Fig. 1) was based on the results of earlier studies (Roggeman et al., 2013; Nawrot et al., 2006; Waegeneers et al., 2008). The most important metals that polluted these regions in the past were Zn, Cd, Pb, As and Co. All cows from polluted sites resided in one of these regions. Regions with normal background levels of metals were selected for reference samples.

2.2. Collection of the samples in the slaughterhouse

From each study animal, samples were taken by an officially appointed veterinarian from the Belgian Federal Agency for the Safety of the Food Chain (FASFC) in the slaughter house. All samples were taken by using the same protocol.

At least 5 g of muscle was cut off at the neck of the cow. From every liver, the left liver lobe was cut off and put into a plastic bag. Every right kidney was collected and put into a plastic bag. From every right lung, two samples were taken with a stainless steel scalpel. The cranial and caudal lobe were cut off and put in separate plastic bags. This separation in cranial and caudal lung part was used to be able to test a new theory to study if some metals are significantly absorbed into the body via the olfactory air-lung pathway. It was assumed that when metals where inhaled, their concentrations would be higher in the upper (cranial) lung lobe compared to the lower (caudal) lung lobe.

From every study animal, the sanitel number was used to label the samples and a copy of the passport was stored. All samples were immediately frozen and stored in a freezer (−20 °C) until analysis.

2.3. Homogenization and preparation of the samples

All tissues (kidney, liver, lung, muscle) were trimmed of fat and homogenized using a Santosafe blender with a 2 L polycarbonate jar and stainless steel blade, to avoid contamination. After every sample the jar and blade were washed with Mili-Q water and detergent to degrease the jar and then again washed with milli-Q (Millipore®, Brussels, Belgium) and dried with lab paper. When metal concentrations are measured in kidney, in most studies only cortex tissue is used because it was already proven to accumulate more metals than medulla in for rats (Southard and Nitisewojo, 1973) and humans (Bush et al., 1995; Friberg et al., 1986; López-Artíguez et al., 1995). To test if this is also justified for cattle different subsamples of every kidney were taken for metal measurements. Therefore one subsample of pure cortex and another of pure medulla were dissected with a stainless steel scalpel before homogenizing the whole kidneys (cortex and medulla). When metal concentrations in small animals are measured the organs are small and metals are measured in the homogenized tissue of the whole organ. But the liver and kidney of a cow is more than 1 kg and as a result homogenizing the whole organ is very difficult, time consuming and labor intensive. Therefore it was also tested if it is necessary to homogenize the whole organ in order to get correct metal measurements. Therefore from 16 livers a non-homogenized subsample was dissected before homogenization. All samples (±0.2 g ww) were put in preweighed falcon tubes, reweighed and were oven dried at 60 °C for 96 h. Thereafter all dried samples were weighed and digested for metal analysis.

2.4. Measurements

2.4.1. Metal measurement

The different tissues were digested with nitric acid (69%, Pro analysis, Merck) and hydrogen peroxide (30%, Pro analysis, Merck) (HNO_3/H_2O_2 as ratio 3/1) followed by a stepwise open microwave digestion (Blust et al.,

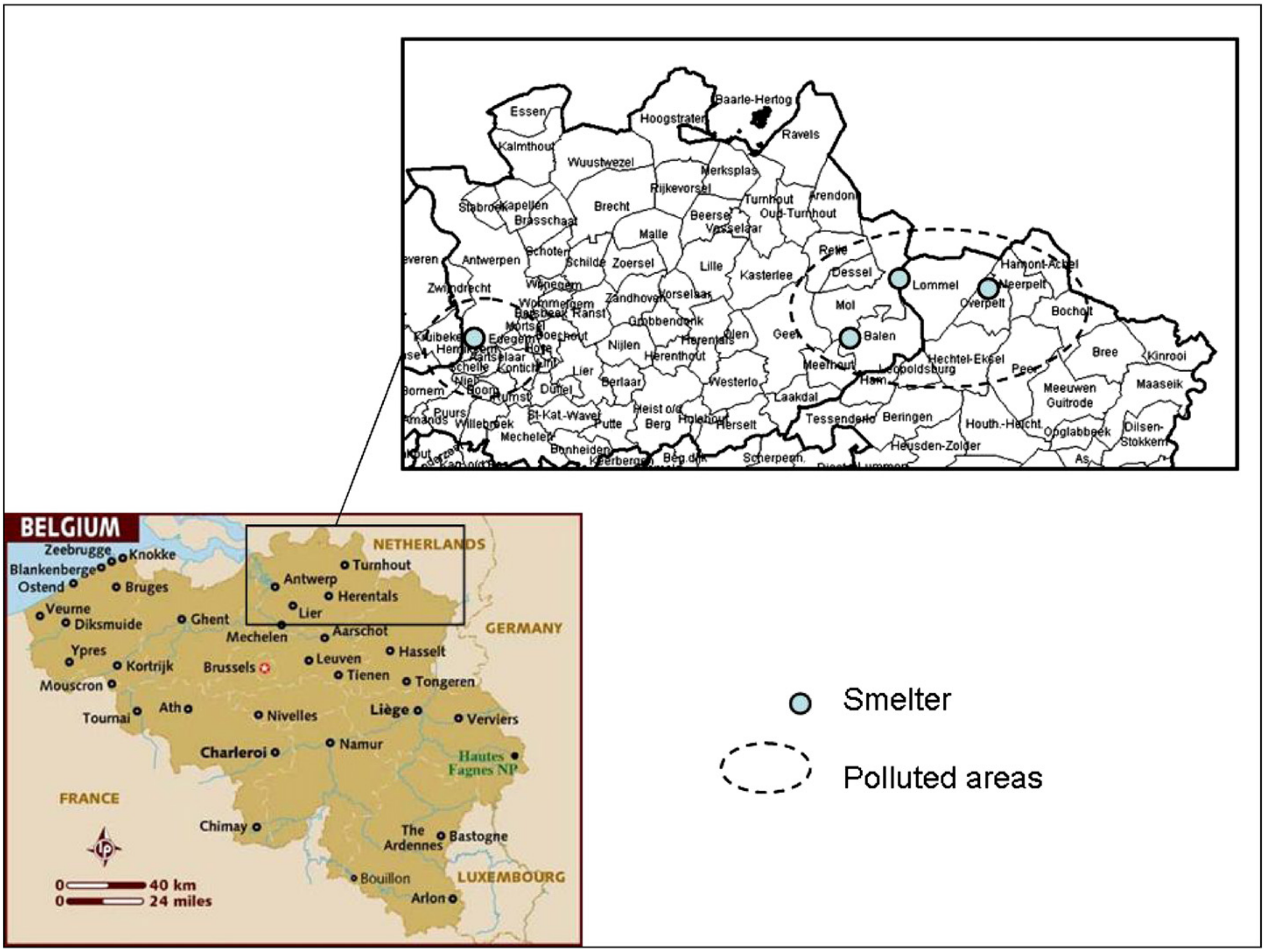

Fig. 1. Location of the polluted areas and smelters that caused the metal pollution.

1988). Before measurement, all samples were diluted with Milli-Q water (Millipore, Bedford, MA, USA) up to 3–6% acid. The concentrations of Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were measured using a high resolution inductive coupled plasma mass spectrometer (HR–ICP-MS, Element XR, Thermo Scientific, Finnigan element 2, Bremen, Germany). All metal concentrations were calculated on their wet weight basis. Analytic accuracy was verified by the use of blanks and certified reference materials (Bovine liver BCR 185R) of the Community Bureau of Reference (Brussels, Belgium). At least two blanks and two reference samples were included for each batch of 40 tissue samples.

2.4.2. Metallothionein measurement

From each homogenized liver and kidney a subsample of 0.2 g was put in an eppendorf tube and homogenized on ice in Tris–HCl buffer (10 mM Tris–HCl, 86 mM NaCl, pH 7.4) at 4 °C with an Ultra-turrax T3 homogenizer (IKA, Labor Technique, Shaufer, Germany). Tissue homogenates were centrifuged at 16,000 *g* for 20 min to isolate the cytosolic fraction (supernatant). Supernatant aliquots were stored at −80 °C until further analysis. Total (Cd, Cu, Zn)–metallothionein concentrations in the supernatant were determined by the ^{109}Cd-labeled saturation method (Klein et al., 1990, 1994), using the Cd-chelex assay (Bartsch et al., 1990). In brief, oxidized metallothionein molecules (MT) were reduced with 2-mercaptoethanol, using Zn^{2+} as an electron donor. High molecular weight proteins were denatured with acetonitrile, while MT-bound Cu was removed with ammonium tetrathiomolybdate. After removal of excessive tetrathiomolybdate and its Cu-complexes with DEAE-sephacel (Sigma, St-Louis, MO USA) the apoprotein was saturated with ^{109}Cd-labeled $CdCl_2$ solution (1 mM, 740 kBq/mL, specific activity). Excessive ^{109}Cd (II) was bound to Chelex-100 (Bio-Rad, Munich, Germany) and removed by centrifugation at 8000 *g* for 5 min. Finally the ^{109}Cd (II) bound to MT in the supernatant was counted during 1 min. in a 'Wizard 3' 1480 Automatic gamma counter (Perkin Elmer, Zaventem, Belgium). The MT concentration was calculated assuming a molar ration of Cd/MT of 7 (Kito et al., 1982).

2.4.3. Histopathological investigation

In addition, at the time of tissue sampling, representative tissue sections of kidney- (cortex and medulla), liver-, muscle- and lung were collected from 4 Galloways from polluted and 3 Galloways from unpolluted sites. The tissues were immediately fixed in 4% formalin and routinely processed for paraffin embedding. Tissue sections of 4 μm were cut and stained with hematoxylin eosin for routine microscopic examination. The liver was evaluated for evidence of hepatocellular damage visible as hepatocellular swelling (cytoplasmic vacuolation) and nuclear changes (hyperchromasia, binuclear cells), as well as general architectural changes such as fibrosis, periportal or centrolobular, and evidence of inflammatory infiltrate. The kidney was evaluated for evidence of tubular epithelial damage visible as cell swelling and pigmentation, glomerular changes (sclerosis) and general architectural changes such as interstitial fibrosis and inflammatory infiltrate. The lung was evaluated for evidence of alveolar epithelial or bronchial epithelial damage and interstitial changes such as fibrosis or inflammatory infiltrate. The muscle

was evaluated for acute or chronic myocyte damage or interstitial fibrosis and inflammation. All lesions were scored as mild (if minimal change was present compared to the normal tissue), moderate (if more pronounced changes were present compared to the normal condition) or severe (if the changes were significant compared to the normal condition).

2.5. Statistical analysis

All statistical analyses were done using the SAS institute 9.2 software. Before starting, all data were tested for normality using the Shapiro–Wilk test. The confidence interval of 95% was used to evaluate all tests. A multiple regression test was used to determine possible linear relationships between MT concentrations and metal concentrations in liver and kidney. To validate the multiple regression analysis as correct, the residuals had to be normally distributed. Differences in multiple regression between Galloways and dairy cows were evaluated by the significance of their interaction effect, using a mixed model procedure. A nested ANOVA with post hoc Tukey test (using a mixed model) was used to determine differences between the metal concentrations in the different kidney tissues (cortex, medulla and homogenized sample). A Wilcoxon signed rank sum test was used to evaluate possible differences in metal concentration between caudal and cranial lung tissue of each animal. A nested ANOVA (mixed model) was used to test if the MT and metal concentrations in the different tissues differed between Galloways and dairy cows or only within a cow breed, between polluted and unpolluted sites.

3. Results & discussion

3.1. Tissue selection and preparation methods

Homogenization did not affect metal concentration of liver samples (Fig. S1). This suggests that metals are uniformly divided through the liver tissue of cattle. This is good news for future studies because homogenizing these large organs is very time consuming and labor intensive. For all metals, except Ag, Cr and Ni, the metal concentrations were significantly higher in cortex samples than in medulla samples (Fig. 2). The concentrations in the homogenized whole kidney samples were also significantly lower than those in the cortex samples but in a lesser extent. When future monitoring studies for cattle are conducted to measure total metal concentration it is therefore recommended to use only cortex tissue. This observation of a higher concentration in the kidney cortex is similar to observations for humans and other animal species (Bush et al., 1995; Holterman et al., 1984; Nordberg et al., 1979). The results also suggest that homogenization of the cortex tissue seems not necessary.

3.2. Metal concentrations in the different tissues

Differences in uptake and accumulation between different metal types were observed between the different internal tissues. When the concentrations were compared between all different tissue types, the Cd, Pb, As, Cr and Al concentrations were the highest in kidney tissue while for Ag, Mn, Co, Ni and Cu the highest levels were found in liver tissue, for Zn the highest levels were found in muscle tissue and for Fe the highest levels were found in lung tissue (Fig. 3 and Table S2). The mean Cd concentrations in the liver (0.524 ± 0.0996 μg/g ww) and kidneys (4.32 ± 0.792 μg/g ww) of all sampled cows during the present study were 4 to 15 times higher compared to those found in other European countries such as Poland (Włostowski et al., 2006), Spain (López-Alonso et al., 2004), Sweden (Jorhem et al., 1991), Finland (Tahvonen and Kumpulainen, 1994) and the Netherlands (Spierenburg et al., 1989). This is probably due to a lower environmental pollution level of those other countries. The Cd, Pb, Cu and Zn concentrations in liver, kidney and muscle were similar to those of an earlier study in Belgium (Waegeneers et al., 2008) from the same region. The Zn and Cu concentrations in liver and kidney and muscle of all cows were comparable to those found in other studies from polluted sites (López-Alonso et al., 2000; Spierenburg et al., 1989). All As and Pb levels in the livers and kidneys were within the normal range for animal health purposes (Table 1). Most renal and hepatic Cd concentrations were within the high range for bovine livers and kidneys. Based on current knowledge, exposure at this level is not associated with adverse effects in terms of animal health and performance (Puls, 1994). For Ag, Mn, Co, Cu, Zn

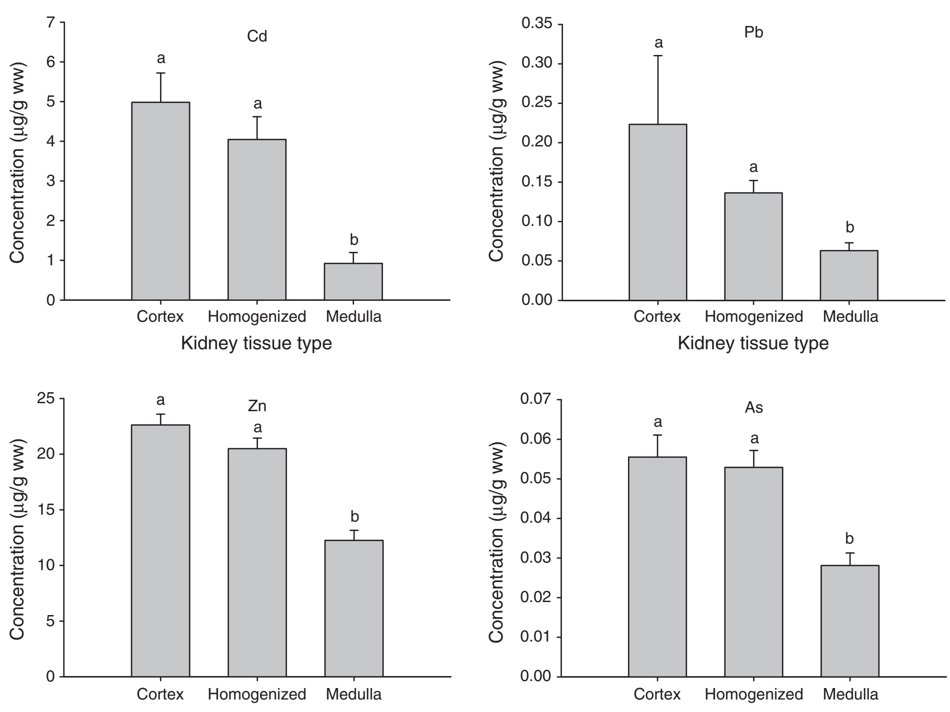

Fig. 2. The differences in metal concentration between cortex, medulla and homogenized total kidney sample. Different letters mean they are significantly different from each other.

and As the concentrations in the different tissues were significantly higher in the sampled Galloways than in the sampled dairy cows. The Cd and Pb levels were significantly higher in tissues of cows from polluted sites (Table 2). In general, within the sampled Galloway group, the internal metal concentrations were more often significantly higher in cows from polluted areas. In the sampled dairy cow group the differences between internal metal concentrations in cows from polluted and unpolluted sites were less often significant. These results show more differences in internal metal concentrations between the 2 cattle breeds than between polluted and unpolluted sites. This is in a way unexpected because the pollution gradients on which the study animals were selected were quite similar for both breeds. This result can suggest that Galloways accumulate more metals than dairy cows, but the most plausible explanation can probably be found in the differences in lifestyle and diet of both cattle breeds. Primarily, unlike semi-wild living Galloways, dairy cows on farms do get extra feed enriched with essential metals. Secondly dairy cows graze on pastures only during spring and summer and stay inside the stables during winter while Galloways were grazing year round in nature reserves without additional feeding. This is similar to other studies with dairy and beef cattle which suggested that the differences in metal accumulation could be associated with a higher metal dietary intake and a higher hepatic metabolism associated with milk production. (López-Alonso et al., 2003; Waegeneers et al., 2008).

3.3. The relevance of olfactory metal absorption

A distinction between cranial and caudal lung tissue was used during this study to test a new theory. This theory assumed that when metals had entered the body via olfactory absorption the levels would probably be higher in the cranial parts of the lung. When they would only have entered via oral intake and absorption into the bloodstream the levels were assumed to be higher in the caudal lung parts (via only the blood–lung pathway) or to be equally divided

Table 1
Normal, high and toxic (chronic) concentrations on arsenic, cadmium and lead in bovine livers and kidneys in ppm (mg/kg) wet weight for animal health purposes. Table adapted from Puls, 1994.

	Normal	High	Toxic
Arsenic (As)			
Liver	0.004–0.4	1.0–50	7.0–100
Kidney	0.018–0.4	1.5–5.0	5.0–53
Cadmium (Cd)			
Liver	0.02–1.0	1.4–9	50–160
Kidney	0.05–1.5	5.0–36	100–250
Lead (Pb)			
Liver	0.1–1.0	2.0–10	5.0–300
Kidney	0.2–2.0	3.0–20	5.0–700

in both lung parts. For all cows from polluted areas the mean cranial Cd concentration was significantly higher than the mean caudal concentration (Fig. 4). This suggests that there is an important part of the Cd intake via olfactory absorption. This was also found for humans living in the same areas as the cows of this study, where inhalation of Cd was found to increase the risk of lung cancer (Nawrot et al., 2006). For Galloways and dairy cattle from the polluted sites the cranial Mn concentration was significantly higher than the caudal concentration which suggests that Mn is also partially taken up via inhalation (Fig. 4). For dairy cattle from polluted sites the mean Pb concentration was significantly higher in caudal than in cranial lung tissue. For all Galloways and dairy cattle the caudal Co concentrations were significantly higher than the cranial part of the lungs. For the other metals there was no significant difference in concentration between caudal and cranial lung tissue. These results suggest that except for Cd and Mn the absorption by the respiratory system is probably negligible. Because this theory has not been studied before, a more detailed analysis is recommended for future investigation,

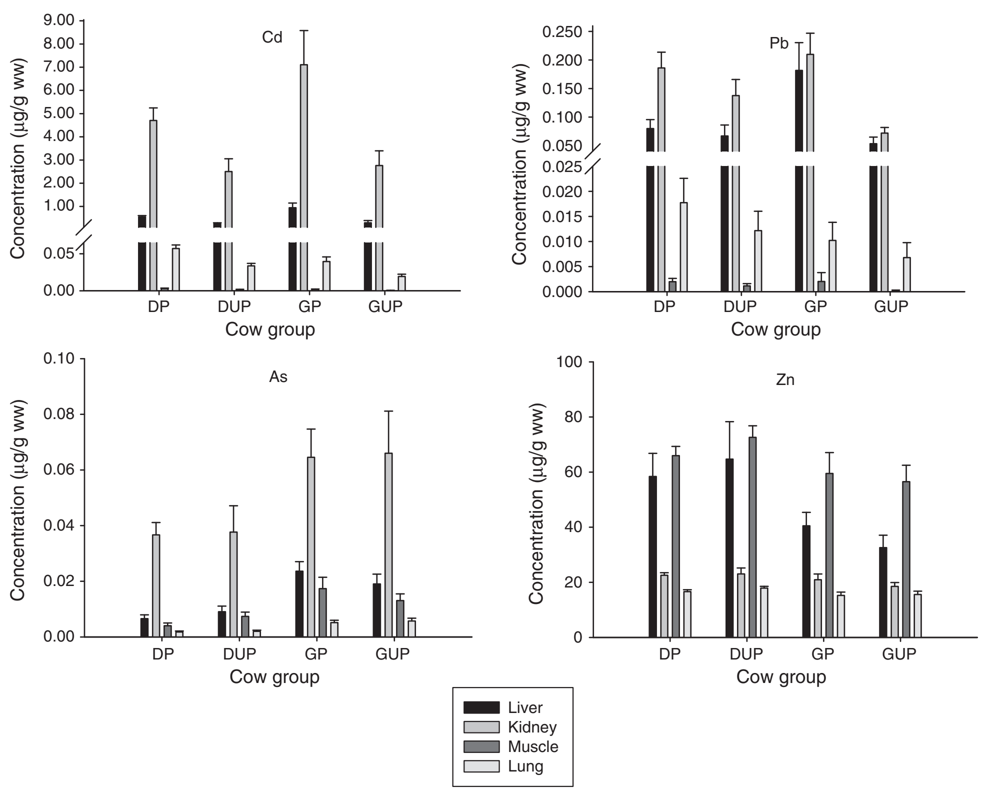

Fig. 3. The mean Cd, Pb, As and Zn concentrations in liver, kidney, muscle and lung tissue (cranial and caudal together), with their SE. The 4 different cow groups are: DP = Dairy cows from polluted areas, DUP = Dairy cows from unpolluted areas, GP = Galloways from polluted areas, and GUP = Galloways from unpolluted areas.

Table 2
Significant differences in metal concentrations between the 2 cow breeds and, within each cow breed, between polluted and unpolluted sites (nested two-way ANOVA, on LOG transformed data). Only significant p-values ($p < 0.05$) or trends ($0.1 < p < 0.05$) are given.

	Significant differences between:	Ag	Cd	Pb	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
Liver	Galloways and dairy cows						<0.0001				<0.0001	0.0035	<0.0001
	Polluted and unpolluted sites	0.003	<0.0001	0.0095									
Kidney	Galloways and dairy cows	0.0002				0.0137			0.0019		<0.0001	0.0261	0.0079
	Polluted and unpolluted sites		<0.0001	0.0025					0.0017				
Muscle	Galloways and dairy cows	<0.0001		0.256			0.0388	0.0125		0.0121		0.0189	0.0015
	Polluted and unpolluted sites		Trend										
Lungs	Galloways and dairy cows	0.0047	0.0004		0.0028			<0.0001	0.0261		<0.0001	Trend	0.0003
	Polluted and unpolluted sites		0.0082						Trend				

thereby taking into account the possible importance of particle size distribution of the inhaled particulate matter (PM). Earlier research (Ray and Lahira, 2010) suggests that particle size distribution significantly affects particle deposition in the respiratory tract. Coarse particles are deposited almost exclusively in the nose and throat, whereas fine and ultrafine particles generally are able to penetrate to deep areas of the lung. If metal concentrations would be higher in ultra fine and fine PM, it would be possible for metal deposition from PM inhalation to result in higher concentrations in the lowest (caudal) portions of the lungs. In that case our theory should also take into account the particle size of the inhaled metals.

3.4. MT levels and metal binding capacity

The mean MT concentrations were significantly higher in liver (23.8 ± 3.38 nmol MT/g tissue) than in kidney (9.16 ± 0.930 nmol MT/g tissue), (Wilcoxon signed rank sum test, $p < 0.0001$). A two way ANOVA with post hoc Tukey test showed that the MT, Zn and Cu concentrations in the liver differed significantly between Galloways and dairy cows although the Cd concentrations only differed between polluted and unpolluted sites, within each cattle breed. In the kidneys the Zn and Cu concentrations differed significantly between Galloways and dairy cows while the MT and Cd concentrations only differed between polluted and unpolluted sites, within each cattle breed (Fig. 5.; nested ANOVA).

The theoretical MT concentrations were calculated based on the measured metal concentration and their metal-specific number of binding cites on the MT. For Cd and Zn there are 7 binding sites on an MT protein and for Cu there are 12. The mean measured MT-concentrations in liver and kidney were much lower than the mean theoretical MT concentrations that would be needed to bind all Cd, Cu and Zn (Fig. 6). The MT concentrations in both liver and kidney of all cows were sufficient to potentially bind all renal and hepatic Cd. On the other hand, the MT concentrations were not sufficient to also bind all Cu and Zn. Although the binding affinity of MT is the highest for Cd followed by Cu and is lowest for Zn, this does not necessarily mean that there will be no Cu or Zn bound to MT when there is still free unbound Cd in the organ, or that Cd will displace all Cu and Zn from the MT. It is therefore impossible to predict the exact detoxification capacity of the cows but it can provide an idea about a certain potential. Therefore it seems more appropriate to refer to it as the "potential" detoxification capacity of the cows. The potential detoxification capacity in the organs of the cows from the present study seems insufficient to bind all measured Cd, Cu and Zn. If excessive Cd, Zn or Cu in the organs cannot be neutralized by binding to MT's it can cause renal or hepatic damage (Nordberg and Nordberg, 2009), and may even cause death (López-Alonso et al., 2005). On the other hand also other metal binding proteins such as glutathione can play a role in the metal detoxification processes of the organs (Ballatori, 1994; Gharieb and Gadd, 2004).

3.5. Relationship between MT concentration and metal concentrations in kidney and liver

Based on the results of the multiple regression analysis between the MT and the Cd, Zn and Cu concentrations (in μg/g ww, $n = 59$), Eqs. (1) and (2) could be created to describe the relationship between MT and metal concentrations in kidneys and livers of cattle. For this analysis only the metal concentrations from the homogenized kidney samples were used because the MT concentrations were also measured in homogenized samples. There were no significant differences in the MT–metal relationship between Galloways and dairy cows (ANOVA with interaction test). Therefore Eqs. (1) and (2), and Fig. 7 apply for both cattle breeds:

$$\text{Liver}: \quad MT = -10.405 + (0.652 \times Zn) \qquad R^2 = 0.92 \quad p<0.0001 \tag{1}$$

$$\text{Kidney}: \quad LOG_{10}(MT) = 0.54667 + (0.58472 \times LOG_{10}(Cd)) \qquad R^2 = 0.41 \quad p<0.0001. \tag{2}$$

For the livers only Zn was significantly linear correlated to the measured MT concentrations while for the kidneys only Cd was

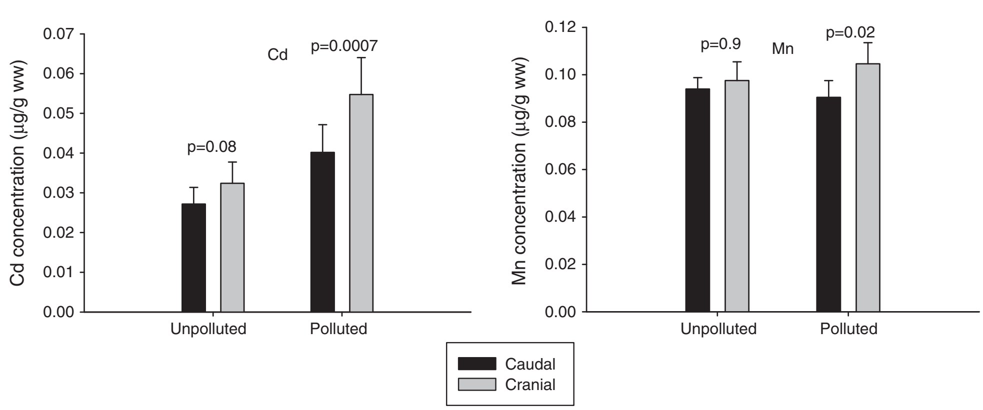

Fig. 4. Differences between metal concentrations in cranial and caudal lung tissue from Galloways and dairy cows from polluted and unpolluted sites.

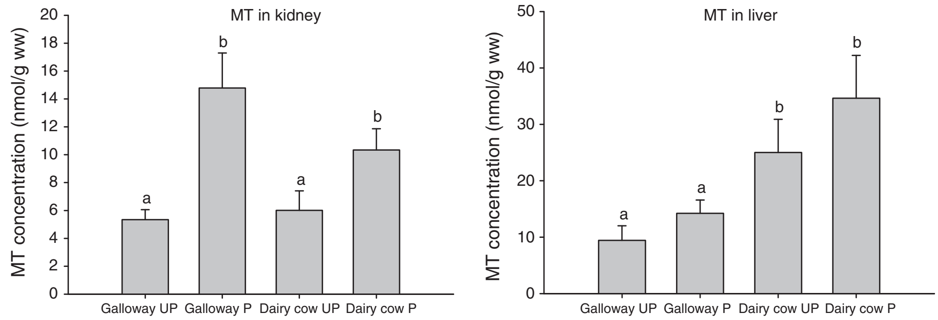

Fig. 5. The mean MT concentrations with SE, measured in the kidneys and livers of Galloways and dairy cows from polluted (P) and unpolluted (UP) sites. Different letters mean they are significantly different.

significantly correlated with the measured MT concentrations. In both organs no significant relations were found between the Cu and MT concentrations. These results suggest that MT's in the kidneys of cows are mostly induced by Cd and in the liver by Zn. The renal Cd concentration described 41% of the variation in MT concentrations in kidneys while the hepatic Zn concentrations described 92% of the variation in MT concentrations in livers. This result is comparable to a study of López-Alonso et al., 2005, where also a direct correlation was found between the Zn and MT concentrations in the livers of Galician cattle.

3.6. Histopathological investigation

In all livers from Galloways from polluted sites a mild vacuolar degeneration and ad random mild hepatocellular necrosis was observed (Fig. 8). This was not observed in livers from Galloways from unpolluted sites. Similar histopathological lesions have been reported in livers from calves and other animals in studies with metal poisoning (Brzóska et al., 2003; Damek-Poprawa and Sawicka-Kapusta, 2004; Horký et al., 2002; Jadhav et al., 2007; Nakazato et al., 2008). On the other hand this type of lesion is not specific for metal poisoning only and thus other underlying causes cannot be ruled out with certainty. This result suggests that the high hepatic metal concentrations in liver together with the MT deficiency to bind all metals possibly caused light hepatic damage, but this needs further investigation in the future.

Although the Cd and Pb concentrations in kidneys were high, no histopathological damage was observed during this study although in studies with comparable Cd concentration, in kidneys from horses and red deer, ranging between 20 and 100 μg/g wet weight, fibrosis and arterial constriction have been described (Elinder et al., 1981; Fowler et al., 1975; Friberg et al., 1974). In other studies chronic Cd exposure was also found to damage primarily the kidneys, causing tubular degeneration, interstitial inflammation, apoptosis and glomerular swelling (Groten et al., 1994; Liu et al., 1998a; Nicholson et al., 1983). Importantly, during chronic exposure, the Cd-induced nephrotoxicity is dependent on the renal Cd concentration. Several studies suggest differences in Cd thresholds that cause renal injury among mammals and birds. Still, the reason for this difference in susceptibility to Cd toxicity is not known and remains to be elucidated (Larison et al., 2000; Liu et al., 1998b; Satarug et al., 2003; Włostowski et al., 2010).

3.7. Human health risks

The European maximum standards for Cd levels in cattle tissue are 0.05 μg/g, 1 μg/g and 0.5 μg/g fresh weight in respectively muscle, kidney and liver. For Pb they are 0.1 μg/g, 0.5 μg/g and 0.5 μg/g fresh weight in respectively muscle, kidney and liver (EU, 2006). During the present study, 40% of all livers (44% of all Galloway and 38% of all dairy cows) exceeded this maximum consumption standard for Cd and 1 liver of a Galloway cow exceeded the maximum consumption standard for Pb. Of all kidneys 85% (91% of all Galloways and 81% of all dairy cows) exceeded the maximum consumption standard for Cd and 1 kidney of a dairy cow exceeded the maximum consumption standard for Pb. As a result of the high Cd levels in kidneys measured during earlier studies, the Belgian Food Safety Authority (FAVV) did already exclude all bovine kidneys from the human food chain. All Cd and Pb levels in muscle were far below the European maximum level and were comparable with the results of a similar study in the same region (Waegeneers et al., 2008) and within previously reported ranges in other countries (Jorhem et al.1991, Langlands et al., 1988; López-Alonso et al., 2000; Olsson et al., 2001). When all sampled cows were classified only based on the pollution of the area of which they came from, the renal Cd levels of all sampled cows from polluted areas exceeded the European max. level compared to 74%

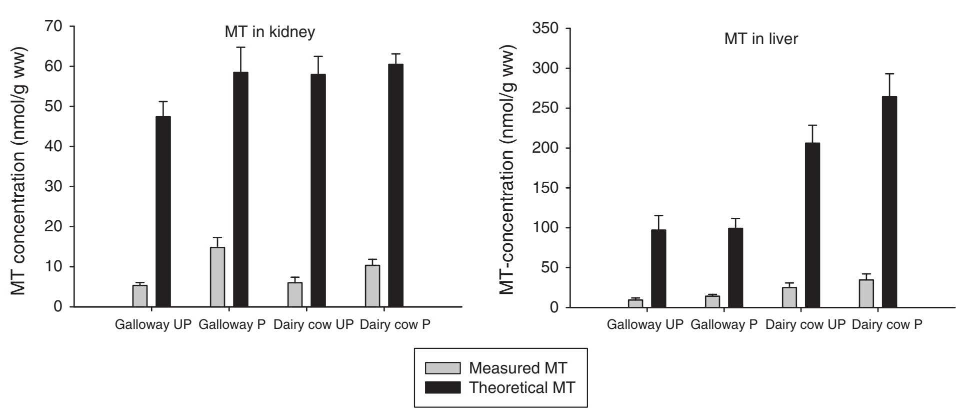

Fig. 6. The mean measured hepatic and renal MT concentrations compared with the theoretical MT-concentrations that are needed to bind all measured Cd, Cu and Zn for Galloways and dairy cows from polluted (P) and unpolluted (UP) sites.

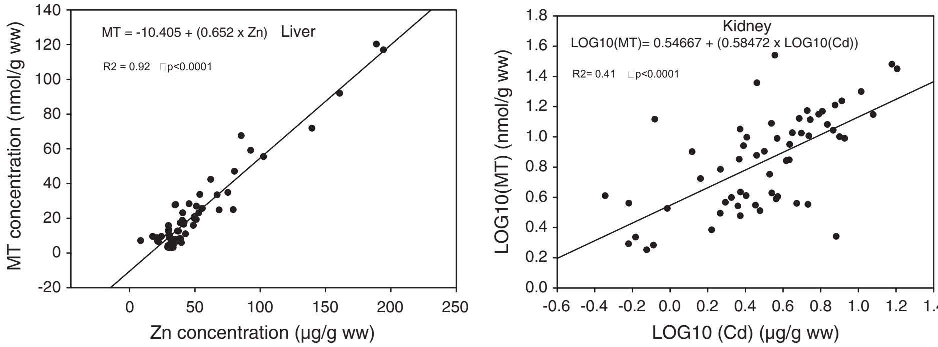

Fig. 7. Linear regression between the hepatic MT and Zn concentration and the renal MT and Cd concentration.

of all sampled cows from unpolluted areas. The hepatic Cd levels of 75% of all sampled cows from polluted areas exceeded the European maximum level compared to 3% of all sampled cows from unpolluted areas. This suggests that it can be useful to take into account the pollution background of the region where the slaughtered cattle are from, when risks for human consumption are considered. The Cu and Zn levels in livers of all sampled dairy cows (between 14.9 and 142 µg/g fresh weight for Cu and between 29.7 and 195 µg/g fresh weight) were high compared to those of all the Galloways and also compared to other studies (Spierenburg et al. 1988; López-Alonso et al., 2000; Cai et al., 2009). The Zn levels in muscle (between 23.2 and 104 µg/g fresh weight) were also high compared to other studies (López-Alonso et al., 2000). Because only a European norm exists for Cd and Pb, the maximum amounts which are recommended to eat without risk for an average person of 70 kg, were calculated for Cd, As, Al, Cr, Co, Cu and Zn, based on the existing minimum risk levels (MRLs) for chronic (or, if not available, intermediate) oral exposure (Table 3). This result suggests that a person of 70 kg should not eat more than 150 g cow meat per day because of the high Cr levels in the muscles of the cows. Also the high Zn levels can be a potential human risk when more than 326 g cow meat is consumed per day.

4. Conclusions

Trace metal concentrations have been measured in destructive and non-destructive tissues of Galloway- and dairy cows from contaminated and reference areas in Belgium. The results of this study suggest that in cattle, metals are homogeneously spread through the liver and are most concentrated in the cortex of the kidneys. Therefore, when cattle organs are studied in the future it is sufficient to use only a small piece of kidney cortex tissue and a small piece of liver tissue. Mixing the whole organ for homogenization is not necessary. For most metals, the cattle management factors such as access to pastures and additionally feeding of cattle seemed to have a more important influence on the internal metal accumulation level of the cows than the pollution gradient of the area where they live. Only for Cd, Pb and Ag the cows from polluted areas had significantly higher internal concentrations, than cows from reference areas. The results of this study suggest that Cd is the most important metal for the MT induction in kidneys and Zn is the most important metal for the MT induction in the liver of cattle. They also suggest that the total MT concentration of the kidneys and livers is probably not sufficient to detoxify all accumulated metals. But only in the liver some light damage was observed during histopathological investigation of the tissue, as a possible result of the high metal accumulation and insufficient detoxification through binding to metallothionein. During this study only for Cd and Mn uptake through olfactory absorption seemed to play a relevant role in the total uptake in cattle. Cadmium concentrations exceeded the European consumption maximum levels in 40% of the livers and 85% of the kidneys from all sampled cows and were significantly higher in the livers and kidneys from cows from polluted areas. Based on the existing minimum risk levels (MRLs) for chronic oral human metal exposure, the present results suggest that a person of 70 kg should not eat more than 150 g cattle meat per day because of the high Cr levels in the

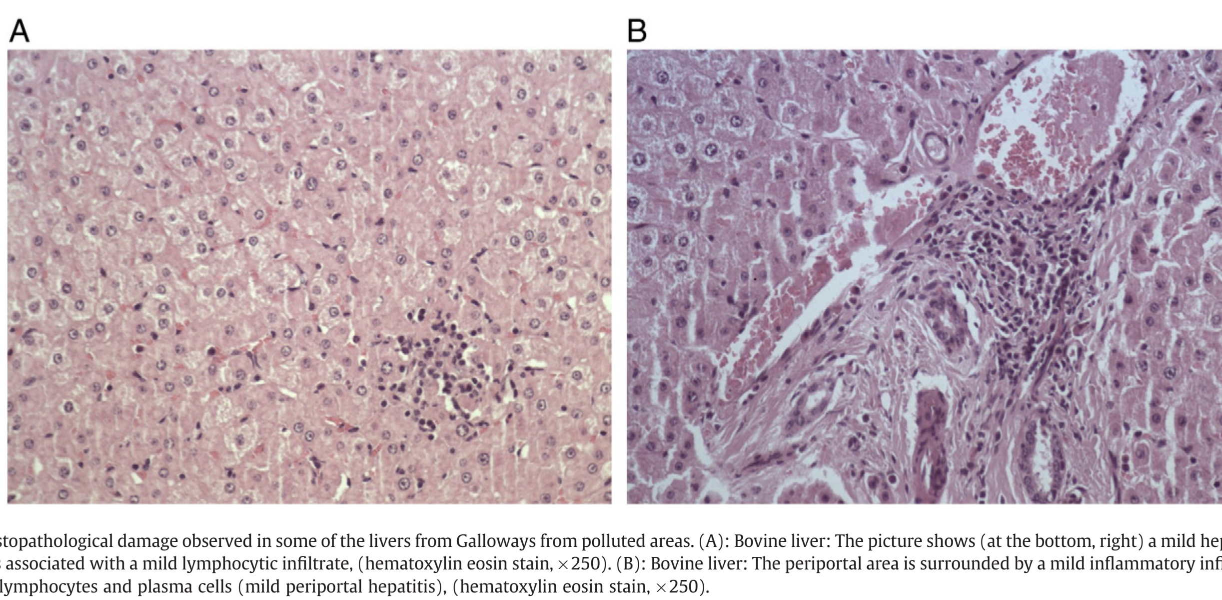

Fig. 8. Histopathological damage observed in some of the livers from Galloways from polluted areas. (A): Bovine liver: The picture shows (at the bottom, right) a mild hepatocellular apoptosis associated with a mild lymphocytic infiltrate, (hematoxylin eosin stain, ×250). (B): Bovine liver: The periportal area is surrounded by a mild inflammatory infiltrate composed of lymphocytes and plasma cells (mild periportal hepatitis), (hematoxylin eosin stain, ×250).

Table 3
Comparison between the MRL's (ATSDR, 2012) and the mean concentrations measured in the muscles (=meat) of all cows.

	Cd	As	Al	Cr	Co	Cu	Zn
MRL (mg/kg/day)	0.0001	0.0003	1	0.0009	0.01	0.01	0.3
MRL (mg/day) for a person of 70 kg	0.007	0.021	70	0.063	0.7	0.7	21
Mean concentration in measured muscle samples (mg/kg)	0.002	0.009	0.214	0.421	0.005	0.667	64.5
Max. edible amount of cow meat per day (kg) for a person of 70 kg	3.5	2.30	327	0.150	140	1.049	0.326

muscles. Also the Zn levels in muscle tissue suggest a restriction of 326 g cattle meat consumption per day.

Conflict of interest

There are no conflicts of interest for the submitted manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.07.007.

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